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EFFECT OF LIGHTING CONDITIONS ON THE CONVERSION OF GREEN PIGMENTS

Ву

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Effect of Lighting Conditions on the Conversion of Green Pigments by

O.G. Sudyna and K.P. Dowbish

Among the porphyrin pigments of higher plants green color is possessed by chlorophyll, chlorophyllide, protochlorophyllide, protochlorophyllide, protochlorophyllide, protochlorophyllide, protochlorophyllide in circle double bonds in the forbine part of the molecule and by the absence or presence of the phytol group. Frotochlorophyllide and protochlorophyll, in contrast to chlorophyll and chlorophyllide, have a double bond in the IV ——pyrrole group between 7 and 8 carbon atoms. Protochlorophyllide and chlorophyllide have an acidous nature through the presence of a free carboxyl group of propionic acid group, which in case of protochlorophyll and chlorophyll is esterified with phytol. Protochlorophyllide, chlorophyllide and protochlorophyll have maximum ratios to the final stage of chlorophyll biosynthesis.

In a previous work (Sudim, 1961) was discussed the modern state of feeding for the direct predecessor of chlorophyll and the necessary additional investigations were conducted on the final stage of chlorophyll biosynthesis for the purpose of finally determining by what actual process all this takes place. On the basis of this we made it our task to investigate the influence of illumination on the transformation of green pigments. We investigated shoots of wheat, which were grown in Fetri vases under various lighting conditions. The pigments were designated with the aid of subsequent utilization of two methods: separation on paper by a previously described by us method (Sudim, 1959) and by determining the absorption spectrum on an AF-4 spectrophotometer. During chromatographic distribution of chlorophyll and protochlorophyll pigments the latter were applied with an eyedropper on paper sufficiently high above the starting spot. Chlorophyllide and protochlorophyllide remain at the

points of application. Chlorophyllide and protochlorophyllide just like chlorophyll and protochlorophyll, are easily distinguishable by the spectral properties. Successive utilization of chromatographic distribution of pigments and the determination of absorption spectra offer the possibility of accurately distinguishing the green pigments of the plants. In our experiments we have not utilized any materials fixing methods. Under dark variants extraction and application of pigments on chromatographic paper was carried out at faint green lighting, which, as has been established, causes no restoration reactions of the IV pyrpole ring.

Investigation of pigments of etiolated wheat shoots revealed, that a predominant majority of green pigments remains at the starting spot.

In place of protochlorophyll was noticed only a perceptible spot (smudge), the color of which could not been established. Given below are two illustrations - one from the chromatogram of pigments of the inner shell of pumpkin seeds of ordinary - Cucurbita pepo L (I), which of the green pigments ---- are contained only by protochlorophyll. the second one - from the chromatogram of pigments of etiolated shoots (II) (Fig.1). The distribution of pigments on the chromatogram immediately brings to mind that the green pigment of eticlated shoots does not have the phytol group, because upon it depends the ability of porphyrin compounds to dissolve in petroleum ether and to be applied with an eyedropper on chromatographic paper. Separation of this phytolless pigment from the spot with the aid of acetone and the determination of this absorption spectrum offer the possibility of considering this pigment as a protochlorophyllide, which has no chlorophyllide admixtures. The absorption spectrum of protochlorophyllide is identical with the absorption spectrum of protochlorophyll, and the absorption spectrum of chlorophyllide is identical with the absorption spectrum of chlorophyll. Between themselves these two pairs of green pigments differ mainly by the absorption maximum in the red zone of the spectrum; chlorophyllide and protochlorophyllide make maximum absorption of rays with a wave length of 625-627 mm.

chlorophyllide and chlorophyll - 660-663 mm. In fig.2 is shown the curve for the absorption spectrum of a pigment, eliuvated from the starting spot by chromatograms of pigments of etiolated wheat.

SEE PAGE 3a FOR PICTURE OF FIGURE 1.

Fig. 1. Photos of Pigment Chromatograms

l-internal shells of pumpkin seeds. II-eticlated wheat (1 g); l-carotyn . 2-xanthophyll; 3- protochlorophyll; 4-starting spot with green phytolless pophyric pigmenst.

T.M.Godnev, N.K.Akulovich (1960) and N.K.Akulovich (1961) have also revealed in etiolated kidney-beans (Phaseolus vulgaris L) among the green pigments close to 85% of protochlorophyllide.

To exposure to light of etiolated plants leads to gradual conversion of protochlorophyllide into chlorophyll. These transformations are not realized in a single act (fig.3). The first ten moments of illumination lead to a considerable reduction in the amount of protochlorophyllide and accumulation of insignificant amount of chlorophyllide.

Table 1 represents gradual accumulation of chlorophyll in etiolated wheat shoots upon exposure to light. In the investigations carried out by N.K.Akulovich (1961), ten minutes light exposure in the etiolated kidney-bean shoots, which have been frozen first, of the total sum of newly formed pigments over 50% are chlorophyllides. Within 30 minutes the amount of chlorophyllide rises; only traces of protochlorophyllide re-

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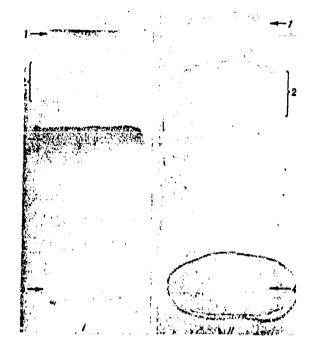


Figure 1. Photos of Pigment Chromatograms (see page 3)

main; the quantity of chlorophyll rises somewhat. On the whole the dynamics of the quantitative relationship (ratio) of protochlorophyllide and chlorophyllide cannot completely disrupt their transformation, because the amount of chlorophyllide in the plant depends not only upon the rate of its formation, but also upon the rate of its transformation into chlorophyll- just as the amount of protochlorophyllide depends to a greater extent upon the rate of its conversion into chlorophyllide. The most important fact is that in light appears chlorophyllide which has not existed in darkness, and protochlorophyllide is not accumulated in larger quantities in leaves upon exposure to light, while in etiolated plants there is a large amount of protochloro-



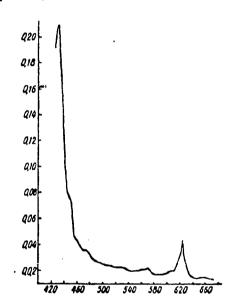


Fig.2. Absorption spectrum of pigments eliowated from starting spot of chromatogram of etiolated wheat.

During the first hour of light exposure
the process of chlorophyll biosynthesis in
shoots becomes gradually normalized and the
conventional relationship between predecessors
of chlorophyll sets in. Protochlorophyllide
in a plant transforms rapidly into chlorophyllide and the latter - into chlorophyll.

Transformation of chlorophyllide into chlorophyll is at a slower rate because in the greening eticlated shoots there is always a certain amount of chlorophyllide. For the next
30 minutes no considerable changes are observed in the spectral chart of the green pigments, only a rise in the amount of chloro-

 chlorophyllide, which transforms into chlorophyllide, and then, as result of chlorophyllase reaction of phytol addition, chlorophyllide transforms into chlorophyll.

The reverse process by which one could anticipate the obtainment upon retardation of a normal process of chlorophyll biosynthesis by the absence of light. Etiolated shoots grown in darkness were placed for two hours in a luminostat with luminescent day light bulbs with a total light intensity of 3500 lux. During this time period, as is evident from fig.4.I. the plants had a small amount of chlorophyllide and an even lesser amount of protochlorophyllide. After exposing to light the plants were again returned into a dark chamber, where they were held for three full diurnal periods (72 hrs). During the first two hours of remaining in darkness the shoots accumulated considerable amounts of protochlorophyllide and they have shown a slight change in the amount of chlorophyllide. This is well evident from fig.4.II. Such a transformation of pigments indicates a considerable retardation in the reaction of protochlorophyllide restoration, which promotes its accumulation, but the reaction of restoration, maybe, continues nevertheless, but at a much lower rate. The latter assumptions are confirmed by data pertaining to the dynamics of chlorophyll amount, and by these investigations (table 2).

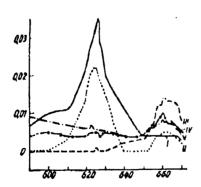


Fig.3.Absorption spectrum of pigments of starting spot from chromatograms 1-etiolated wheat; II after 10 minutes exposure; III-after 30 min_exposure;

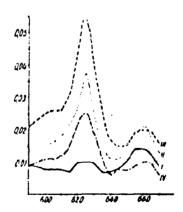


Fig.4. Absorption spectra of pigments of starting spot from chromatograms.
1-etiolated wheat after 2 hrs exposure;
II, III, IV-etiolated wheat, which after 2 hrs

IV-after 60 min exposure; V-after 90 min exposure (pigments with 1.6 g of raw weight in 4.5 cm³ acetone).

exposure remained in darkness for two hrs (II), one day (III), three days (IV) (pigments with 1,6 g of raw weight in 4, cm³ acetone).

within 24 hours (diurnal period) the amount of protochlorophyllide rise considerably in comparison with previous values (fig.4, III). On the third day there is already a noticeable reduction in the amount of protochlorophyllide (fig.4, IV). During the duration of the entire investigation the amount of chlorophyllide decreases only slightly. The amount of chlorophyll on the third day decreases. Most likely, after three days in darkness the rate of initial reactions of chlorophyll biosynthesis decreases; this leads to a change in the amount of protochlorophyllide.

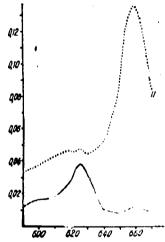
Table 1. Effect of light on the content The investigation results also indicate of chlorophyll in eticlated wheat shoots

	ω.	the retarding influence of light absence
Time of light ex posure (minutes)	Amount of chlorophyl (in mg per l gr of re weight)	entire chlorophyll aw on the rate of biosynthasia pro-
		cess and above all on the reaction of pro-
10	i	tochlorophyllide restoration into chloro-
10 30 60 90	0,002 0,003 0,006 0,009	phyllide. In addition, there is doubt with
	U ₁ UU:9	respect to the possibility of origination
		of restoration reaction on account of the
		power, conserved during the period of light
		exposure. To check these assumptions, were
		conducted special investigations for the
		purpose of studying the influence of light

exposure duration of sticlated shoots on the conversion of pigments in darkness.

It was found, that the time the plants are exposed to light has a deciseve influence on further transformation of pigments in darkness. In fig.5 were plotted two spectral curves, one (I) of which represents the quality status of the pigments in etiolated shoots, which were exposed for two hours to light, and then again for one

day placed in darkness; the other one (II)- represents the quality status of the pigments from eticlated shoots, which differ from the previous ones only by the fact that they were exposed to light for one full day. In shoots, which after two hours of exposure were placed for a full day in darkness, as it was to be expected, were revealed mostly accumulations of protochlorophyllide, and in shoots, exposed to light for a full day, during the following day of darkness accumulated considerable amounts of chlorophyllide and only traces of protochlorophyllide. Even if we compare the spectral curves (fig.6) of pigments of plants, which differ not only by the duration of exposure but also by the period of remaining in darkness, the plants exposed for two hours, again demonstrate a prevalent accumulation of protochlorophyllide per one day in darkness, while plants, illuminated for a full day, even after two days in darkness were abundant in chlorophyllide, which in quantitative ratio by many times exceeds protochlorophyllides.



See page 78 for Fig. 6.

Fig. 5. absorption spectra of pigments from starting spot on chromatograms of eticlated wheat, exposed for 2 hrs to light and after this spent one day in dark and then placed for one day in darkness ness (I) and sticlated wheat exposed for one day andthen placed for one day in darkness (II); (pigments with 2.0 g raw weight in 4.5 cm acetone)

Fig. 6. Absorption spectrum of pigmonts from starting spot on chromatograms of eticlated wheat, exposed for 2 hrs to light (I) and eticlated wheat exposed for one day and then put for two days in darkness (II): (pigments with 2.0 g raw weight in 4.5 om) acetone).

Investigations of the dynamics of the qualitative status of the pigments of sticlated shoots, exposed to light for one day with following three days in darkness

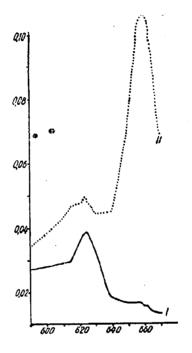


Figure 6. (See page 7)

(fig.7) showed that during the entire time the prevalent pigment is chlorophyllide, and protochlorophyllide shows only traces. When exposed to light for one day etiolated plants accumulated a great amount of chlorophyllide (fig.7.1). In preportion to further remaining in the darkness the amount of chlorophyllide decreases gradually, but during the entire time of its examination it remains a predominantly green phytolless porphyric pigment (fig.7.11.111. IV).

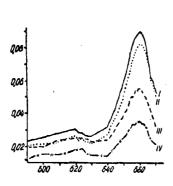
Restoration reactions, possibly, do take place normally in darkness in eticlated plants, which have been exposed to light for a day, otherwise there would have been an accumulation of protochlorophyllide. Next chlorophyllide, most likely, as result of fermentation reaction transforms into chlorophyll.

Investigations with verious exposure time revealed no chlorophyllase activity in etiolated shoots within a day of repeated remaining in darkness, as in variants, which have been exposed to light before them for two hours and a whole day. Cradual reductions in the amount of chlorophyll in darkness without a proper considerable rise in the amount of protochlorophyllide, possibly, indicate uniform retardation of the entire process of chlorophyll biosynthesis. The idea arises, that chlorophyllide can be formed in darkness on account of the energy conserved at the time the plants have been exposed to light.

Determination of the absorption spectrum of a pigment, which remains in the spot on the chromatogram of pigments of green wheat shoots, grown in light showed a great quantitative prevalence "of chlorophyllide as compared with protochlorophyllide and in general the presence of a relatively very large amount of chlorophyllide (fig.8,1). The absence of light for a period of three days does almost not affect the qualitative composition of porphyric pigments of green plants (fig.8,11). At the cutset of being placed in darkness vegetations, which before that were grown in conditions of normal illumination, practically did not change the amount of accumulated chlorophyllide.

For a period of 24 hrs its content also remains practically unchanged under the very came conditions.

Further stay in darkness is accompanied by a reduction in the amount of chlorophyllide in the shoots.



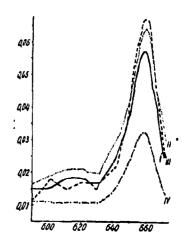


Fig. 7. Absorption spectra of pigments of starting spot on chromatography of eticlated wheat, which was exposed to light for one day (I), and then for two hrs (II), one day (III) three days (IV) and then again placed in darkness (pigments with 0.4 g raw weight in 4.5 cm² acetone).

Fig. 8. Absorption spectra of pigments on starting spot from chromatograms of wheat grown in light (I). after 2 hrs (II). one day (III). three days (IV) stay in dark ness (pigments with 0.2 g of raw weight in 4.5 cm³ of acctone).

protochlorophyllide because of its insignificant

Table 2. Influence of darkening in the content of chlorophyll in etiolated wheat the plants is practically impossible to reveal shoots after 2 hr light exposure

Variant of in vest ig.			f chlorophyll l g of raw w	_
11 -54	Test	I	Test II	Investigations carried out by us have
on light				
				again confirmed the ideas about the influence
2 hrs	1			
		0,016	0.028	of previous illumination on the ability of
after exposure	•		i	•
in darkness.	ĺ		; ;	plants to restore protochlorophyllide
0 5		0.622	0,035	An abbambababba ta danta
2 hrs		0,026 0.019	0,033	to chlorophyllide in darkness.
1 day	1	0,019	0.019	
3 da ys.	1		I .	The investigation material listed here

forresponds with the recently established fact concerning the formation of chlorophyll in darkness (Godney, Rotfarb, 1958, 1960; Godney, Rotfarb, Shlik 1960). The fact is when the absence of light retards mainly the reaction of restoration of the IV pyrrole ring

then the conditions, which are favorable for this reaction, must assure the possibility for the formation of chlorophyll. This formation of chlorophhyl, probably, can always be observed in darkness in direct dependence upon time of previous exposure of plants to light. These observations are in conformity with the data by T.M.Godnew; R.M.Rotfarb; A.A.Shlik (1960) and T.M.Godnew and R.M.Rotfarb (1960) on predominant localization of Clim in the absence of light in the phytol part of the chlorophyll molecule.

In plants, which for a long period of time remained exposed to light, is formed a certain amount of chlorophyllide, which in darkness rather transforms into chlorophyll then having the synthesis of the forbine particle of the pigments. Such a way of transforming the pigments can be observed only under a condition of previous considerable accumulation of chlorophyllide. As soon as the plants remain exposed for a brief time interval to light, chlorophyllide does practically not accumulate. In such instances the chlorophyllase reaction of esterification, probably, cannot take place at a greater rate from the general synthesis of chlorophyll particle formation.

Of certain interest is comparison of the results of our investigations with data obtained by N.G.Down. A.A.Krasnovskiy. A.K.Romanovaya, L.M. Vorobieva, E.M.

Pakshinova and Z.A. Terentyevaya (1961). The process of biosynthesizingchlorophyll in etiolated plants, exposed to light, as is evident from our report, becomes gradually normalized within a period of one and one half to two hours; according to data of above listed authors, the noticeable difference between the amount of C¹⁴O₂ is fixed during light exposure and in darkness and it appears only after two hours of illumination, exactly when chlorophyll is already formed with normal fluorescence of 670-672 mm.

In investigations carried out at the A.A.Krasnovskiy lab (Litvin, Krasnovskiy, Rikhireva, 1959) in eticlated shoots were revealed two forms of protochlorophyll with a fluorescence of 629 mit and 656 mit. Protochlorophyll 656 mit also found in green leaves which remained in darkness for a period of 1-2 days. This protochlorophyll disappears rapidly when exposed to light, transforming at first into a pigment with maximum fluorescence of 690-697 mit, and then into normal chlorophyll. The pigment of 690-697 mit is also observed in green leaves, which for one-two days remain in darkness. The co-workers of this lab have already previously expressed assumptions that the protochlorophyll 656 mit is protochlorophyllide, and the pigment 690-697 mit is chlorophyllide. When these investigations are compared with ours, then the assumptions of the mentioned authors appears to be confirmed.

In recent years questions have been raised as to the process of formation of chlorophyll in plants, growing all the time under light. Considered as possible is the variability of the final stages of chlorophyll biosynthesis which does depend upon the presence of light. Investigations carried out in various labs (Litvin, Krasnovskiy, Rikhireva, 1959; Kaler. Shlik, 1960; Shlik, Kaler, Podchufarova, 1960) refute these assumptions. The presence of protochlorophyllide in green and in sticlated plants was revealed. F.F. Litvin; A.A. Krasnovskiy; and B.T. Rikhireva determined protochlorophyll by the fluorescence spectrum by the maximum of 623 ma. In green leaves they found only traces of protochlorophyll, its amount rises in derkness, and upon further illumination it decreases rapidly. At the A.A. Shlik lab was established the

presence of protochlorophyllide in green, etiolated and darkened green plants. This is in agreement with our data, but in the above mentioned labs, unfortunately, they have not investigated the ratios of protochlorophyllide and chlorophyllide.

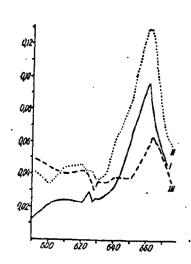


Fig.9. Absorption spectra of pigments of starting spot from chromatogram of wheat control (I), upper part (green) of wheat, treated with streptomycin (II) and its remaining parts (III).

In connection with the investigations by

B.A.Rubin (1959), in which assumptions are
expressed that the inhibiting effect of
streptomycin on the synthesis of chlorophyll
is linked mainly with retardations of reac
tions of protochlorophyll transformation
into chlorophyll, it was interesting to
determine by above mentioned methodon green
phytolless porphyric pigments the albinous
effects of streptomycin treated wheat shoots.
Investigated were pigments of green plants,
green and white batches of streptomycin treat
et plants (fig.9). The green parcels of

streptomycin treated plants, as well as the control plants, have a high amount of chlorophyllide and low amount of protochlorophyllide (fig.9, I.II). Albinous parcels have a considerably lesser amount of chlorophyllide as compared with the green parts (fig.9,III). But chlorophyllide is the predominant pigment even in white parts. It is important to emphasize the unclearness of the maximum on the batch 620-630 mu in both parcels of plants, which have been treated with streptomycin. On the basis of these findings it can, certainly, be considered that streptomycin retards not the reaction of restoration, but the much earlier stages of chlorophyll biosynthesis.

M.Ledoyen, 1957, studying the effect of streptomycin on the accumulations of protochlorophyll in darkness, also arrived at a conclusion regarding the retarding influence of this antibiotic not only on photochemical reaction, but also on the

fermentation reactions connected with the formation of protochlorophyll.

In this way, identification of phytolless green pigments enables to obtain certain information on the final process of chlorophyll biosynthesis and to reveal to a certain extent on what stage the inhibition of chlorophyll formation does take place.

Conclusions

- 1. Investigations do confirm the idea that the final stage of chlorophyll biosynthesis takes place accordance: protochlorophyllide-4chloroph
- 2. In eticlated wheat shoots from porphyrine pigments is accumulated mainly protochlorophyllide.
- 3. In green wheat shoots, which for a day or more were exposed to light, in the darkness the predominant phytol-free porphyrine pigment is chlorophyllide.
- 4. Previous illumination of wheat shoots does affect the ability of the plants in restoring in darkness protochlorophyllide into chlorophyllide. (regeneration)
- 5. To identify porphyrine pigments it is necessary to employ the method of paper chromatography in unison with spectrophotometerings.

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